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(54) **ENHANCED PRODUCTION OF
RECOMBINANT PROTEINS BY TRANSIENT
TRANSFECTION OF
SUSPENSION-GROWING MAMMALIAN
CELLS**(71) Applicant: **NATIONAL RESEARCH COUNCIL
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OF CANADA, OTTAWA (CA)**(21) Appl. No.: **16/552,093**(22) Filed: **Aug. 27, 2019****Related U.S. Application Data**(63) Continuation of application No. 10/477,148, filed on
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2840/203 (2013.01)(57) **ABSTRACT**

Disclosed is a new process for the production of recombinant proteins, by transient transfection of suspension-grown human embryonic kidney cells (293 cell line and its genetic variants) with an expression vector, using polyethylenimine (PEI) as a transfection reagent. In a preferred embodiment, the process uses 293E cells expressing the Epstein-Barr virus (EBV) EBNA 1 protein, in combination with an oriP-based episomal expression vector having an improved cytomegalovirus expression cassette comprising the CMV5 promoter. The process combines in a single step the cell growth, transfection and protein expression, is carried out without changing the culture medium, and allows to achieve high expression levels in a short period of time. The process may be carried out in a serum-free, low-protein culture medium, is easily scalable, compatible with continuous production processes, and fully adapted to high-throughput production of milligram quantities of recombinant proteins.

Specification includes a Sequence Listing.